



Accumulation of perfluoroalkyl substances in human tissues

Francisca Pérez^a, Martí Nadal^b, Alícia Navarro-Ortega^a, Francesc Fàbrega^b, José L. Domingo^b,
Damià Barceló^{a,c}, Marinella Farré^{a,*}

^a Water and Soil Quality Research Group, Dept. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

^b Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, Reus, Spain

^c Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, Emili Grahit, 101, 17003 Girona, Spain

ARTICLE INFO

Article history:

Received 20 February 2013

Accepted 7 June 2013

Available online 25 July 2013

Keywords:

Perfluoroalkyl substances (PFASs)

Human distribution

Tissues

Turbulent flow chromatography coupled to

tandem mass spectrometry (TFC-LC-MS/MS)

Principal Component Analysis

ABSTRACT

Perfluoroalkyl substances (PFASs) are environmental pollutants with an important bioaccumulation potential. However, their metabolism and distribution in humans are not well studied. In this study, the concentrations of 21 PFASs were analyzed in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona (Catalonia, Spain). The samples were analyzed by solvent extraction and online purification by turbulent flow and liquid chromatography coupled to tandem mass spectrometry. The occurrence of PFASs was confirmed in all human tissues. Although PFASs accumulation followed particular trends depending on the specific tissue, some similarities were found. In kidney and lung, perfluorobutanoic acid was the most frequent compound, and at highest concentrations (median values: 263 and 807 ng/g in kidney and lung, respectively). In liver and brain, perfluorohexanoic acid showed the maximum levels (median: 68.3 and 141 ng/g, respectively), while perfluorooctanoic acid was the most contributively in bone (median: 20.9 ng/g). Lung tissues accumulated the highest concentration of PFASs. However, perfluorooctane sulfonic acid and perfluorooctanoic acid were more prevalent in liver and bone, respectively. To the best of our knowledge, the accumulation of different PFASs in samples of various human tissues from the same subjects is here reported for the very first time. The current results may be of high importance for the validation of physiologically based pharmacokinetic models, which are being developed for humans. However, further studies on the distribution of the same compounds in the human body are still required.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of surface-active organic compounds. Because of their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, they have been used for over 50 years in a number of industrial and commercial applications (Zhao et al., 2012). PFASs are highly resistant to breakdown. Therefore, they are persistent in the environment, being able to accumulate in living organisms and biomagnified through the trophic web (Loi et al., 2011; Powley et al., 2008). Moreover, there is a growing concern related to their potentially harmful effects on human health (Vieira et al., 2013). Due to these reasons, the U.S. industry undertook voluntary actions to phase out production of perfluorooctane sulfonic acid (PFOS) between 2000 and 2002, and in 2007 the United States Environmental Protection Agency (US EPA) published the Significant New Use Rules (SNURs) to restrict the production of PFOS and related substances (Lindstrom et al., 2011). Moreover, in 2006, the major PFAS producers committed the Stewardship Program to phase out the global emissions and products containing perfluorooctanoic

acid (PFOA) for 2015. Despite these measures, hundreds of other different PFASs are currently being produced and used. Thus, although the production of PFOA is being phased out by the companies participating in the Voluntary Stewardship Program, environmental contamination and human exposure from PFOA and higher homologue chemicals (e.g. PFNA, PFDA, etc.) are anticipated to continue for the foreseeable future due to a number of reasons: its persistence, their formation from precursor compounds, and the potential for continued production by other manufacturers in the U.S. and/or overseas (Lindstrom et al., 2011).

In 2008, the European Food Safety Authority (EFSA, 2008) established a series of Tolerable Daily Intakes (TDIs) values for PFOS and PFOA at 150 and 1500 ng/kg/day, respectively. PFOS was subsequently included as a persistent organic pollutant (POP) under the Stockholm Convention (UNEP 2010). In 2009, the US EPA Office of Water established the provisional health advisory values for PFOS and PFOA at 200 and 400 ng/L, respectively. It must be highlighted that, although TDIs and the water provisional health advisory were calculated in different basis, in both cases short-term exposure was considered as the relevant period of exposure. This was consistent with PFOA and PFOS toxicity data, which in turn rely upon subchronic exposure experimental values. However, long-term exposures must be considered for the accurate assessment of their potential risk on human health, taking into account that their

* Corresponding author. Tel.: +34 93 400 61 00.
E-mail address: mfarre@cid.csic.es (M. Farré).

presence has been reported in drinking water, ambient air, and food (Domingo et al., 2012a,b; Ericson Jogsten et al., 2012; Ericson et al., 2008, 2009; Post et al., 2009, 2012).

PFASs have been related to different toxicological effects on mammals. In mice, the neonatal exposure to PFOS and PFOA has been linked up to changes in proteins of importance for the neuronal growth and synaptogenesis in the brain developing (Johansson et al., 2009), as well as with neurobehavioral defects and changes in the cholinergic system (Johansson et al., 2008). In addition, perfluorohexanesulphonate (PFHxS) has been related to irreversible neurotoxic effects in neonatal mice, showing a similar behavior to that of other POPs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (Viberg et al., 2013). A recent study in human suggested that higher PFOA serum levels might be associated with testicular, kidney, prostate, and ovarian cancers, and non-Hodgkin lymphoma, according to the concentrations of residents in 6 areas with contaminated drinking water supplies (Vieira et al., 2013).

In the human body, the polar hydrophobic nature of fluorine-containing compounds can lead to increased affinity for proteins (Jones et al., 2003; Luebker et al., 2002; Vanden Heuvel et al., 1992; Weiss et al., 2009). A number of PFASs have been detected in human serum, cord blood and breast milk (Domingo et al., 2012a; Ericson et al., 2007; Fromme et al., 2010; Haug et al., 2009a,b; Llorca et al., 2010). As other bioaccumulative halogenated contaminants (e.g., polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and PCBs), PFASs can have long persistence in the body. However, they do not tend to accumulate in fat tissue. According to outcomes of animal studies, PFOA and PFOS are mostly excreted through the urine (Cui et al., 2010), but limited observations in humans suggest that only one-fifth of the total body clearance is renal (Harada et al., 2005). The elimination half-life of PFOA in humans was roughly estimated to be 3.5 years, while that of PFOS was approximately 4.8 years (Olsen et al., 2007), according to data from retired workers. Post et al. (2012) recently reviewed studies reporting the elimination half-life values between 2.3 and 3.3 years, following an exposure to contaminated drinking water (Post et al., 2012). Information about sources, environmental fate and toxicokinetics of PFOS and PFOA is largely available, while estimation values in the half-lives of PFBS, PFHxS and PFBA (Chang et al., 2008; Lau et al., 2007). In contrast, data on most of the PFASs currently in use, continues to be very limited. It has been hypothesized that the possible harmful effects associated to PFASs accumulation are of special concern during early stages of life (Maisonnet et al., 2012; Post et al., 2012; Schecter et al., 2012). However, their accumulation and distribution in the different human tissues are still poorly understood. The potential accumulation of PFASs with different chain lengths is an issue of great importance for exposure assessment and risk characterization studies. Most current investigations on human accumulation have focused on the occurrence in blood and breast milk, while very few studies have reported levels in other tissues. Kärman et al. (2009) determined the concentrations of six PFASs in liver samples collected post-mortem in Spain. Mean concentrations of 27 and 1 ng/g of PFOS and PFOA, respectively, were found. In turn, Maestri et al. (2006) found levels of 14 ng/g of PFOS and 3 ng/g of PFOA in a pooled liver samples corresponding to seven subjects from northern Italy, while Olsen et al. (2003) reported mean PFOS and PFOA concentrations of 19 and 47 ng/g, respectively, in 30 subjects from USA. Finally, Pirali et al. (2009) detected PFOA and PFOS in thyroid tissue (median levels: 2 and 5.3 ng/g, respectively), concluding that those compounds are not actively concentrated in the thyroid.

The main objectives of the present study were the following: 1) to optimize and validate an on-line analytical approach based on turbulent flow chromatography coupled to tandem mass spectrometry (TFC-LC-MS/MS) for determining PFASs in various human tissues; 2) to measure the levels of 21 PFASs in these human tissues in order to elucidate their distribution and accumulation in the human body. The method optimized for the tissue analysis was carefully selected to accomplish the minimum sample size requirements and to reduce sample manipulation. The analytical procedure was validated for different kinds of tissues, and applied for the

determination of selected compounds in liver, lung, brain, bone, and kidney samples collected post-mortem from 20 subjects. PFASs values were correlated with the concentrations of some heavy metals (unpublished results) in the same tissue samples, as well as with the levels of PCDD/Fs in adipose tissue from 15 of the same individuals (Nadal et al., 2009). To the best of our knowledge, these are the first data reporting the accumulation of a notable number of PFASs in human tissues, as well as comparing the body burden of these pollutants with that of other environmental contaminants (metals and PCDD/Fs).

2. Materials and methods

2.1. Chemicals and standards

Standard solutions were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). The standard analytes used in this study were: i) PFAC-MXB [98% purity in methanol] containing perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTra), perfluorotetradecanoic acid (PFTeA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulphonate (PFBS), perfluorohexanesulphonate (PFHxS), perfluorooctanesulphonate (PFOS) and perfluorodecanesulphonate (PFDS); ii) FTA [98% purity in isopropanol] including perfluorohexyl ethanoic acid (FHEA), perfluorooctyl ethanoic acid FOEA, and perfluorodecyl ethanoic acid FDEA; iii) perfluorooctane sulfonamide (PFOSA) [98% pure in methanol]. Identification and quantification were performed using the following internal standards: i) MPFAC-MXA [>98%] containing [$^{13}\text{C}_4$]-perfluorobutanoic acid (MPFBA ($^{13}\text{C}_4$)), ion [$^{18}\text{O}_2$]-perfluorohexanesulphonate (MPFHxS ($^{18}\text{O}_2$)), [$^{13}\text{C}_2$]-perfluorohexanoic acid (MPFHxA ($^{13}\text{C}_2$)), ion [$^{13}\text{C}_4$]-perfluorooctanesulfonate (MPFOS ($^{13}\text{C}_4$)), [$^{13}\text{C}_4$]-perfluorooctanoic acid (MPFOA ($^{13}\text{C}_4$)), [$^{13}\text{C}_5$]-perfluorononanoic acid (MPFNA ($^{13}\text{C}_5$)), [$^{13}\text{C}_2$]-perfluorododecanoic acid (MPFDoA ($^{13}\text{C}_2$)), [$^{13}\text{C}_2$]-perfluorodecanoic acid (MPFDA ($^{13}\text{C}_2$)), [$^{13}\text{C}_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}\text{C}_2$)); ii) MFTA-MXA [>98%] [$^{13}\text{C}_2$]-perfluorohexylethanoic acid (MFHEA($^{13}\text{C}_2$)), [$^{13}\text{C}_2$]-perfluorooctylethanoic acid (MFOEA($^{13}\text{C}_2$)), [$^{13}\text{C}_2$]-perfluorodecylethanoic acid (MFDEA ($^{13}\text{C}_2$)) and iii) [$^{13}\text{C}_8$]-perfluorooctanesulfonamide (MPFOSA ($^{13}\text{C}_8$)).

Water, methanol, acetonitrile, CHROMASOLV®Plus for HPLC grade, ammonium acetate salt (AcNH₄: MW, 77.08; 98%), and formic acid (HFO) were obtained from Sigma-Aldrich (Steinheim, Germany). To remove possible cross contamination, polypropylene (PP) insert vials and inert taps were used.

2.2. Sampling and pre-treatment

Samples from liver, kidney, brain, lung, and bone (rib) were collected in 2008 from 20 subjects who had been living in different areas of Tarragona County (Catalonia, Spain) at least for the last 10 years. Causes of death were varied, including multiple trauma, subdural hematoma, ischemic heart disease, accident or self-injury. Autopsies and extraction of samples were carried out during the first 24 h after the time of death. Additional data from the subjects, such as age (mean: 56; range: 28–83) and smoking habits information, were collected (Table S1; Supporting Information). Tissue samples were stored at $-20\text{ }^\circ\text{C}$ before analysis. The study protocol was reviewed and approved by the Ethical Committee for Human Studies of the School of Medicine, Universitat Rovira i Virgili, Reus/Tarragona, Spain.

Sample pre-treatment was based on a previously published protocol (Llorca et al., 2010). Briefly, 1 g of each sample was weighed and transferred into a 15 mL PP tube. Then, 2 mL of water were added, and the mixture was shaken. Homogenates were fortified with surrogate

internal standards (to obtain a concentration of each internal standard of 10 µg/L), being digested with 5 mL of sodium hydroxide (20 mM in methanol) during 4 h at 125 rpm on an orbital shaker table at room temperature. After digestion, samples were centrifuged at 4000 rpm, and 20 µL of supernatant were directly injected into the turbulent flow chromatography system.

2.3. Analysis

A turbulent flow chromatograph Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) comprised of a PAL auto sampler (CTC Analytics, Zwingen, Switzerland), two mixing binary pumps (eluting pump and loading pump), and a three-valve switching device unit with six-port valve. The entire system was controlled via Aria software, version 1.6. The on-line enrichment was achieved using a Hypersil GOLD aQ column (2.1 × 20 mm, 12 µm particle size from Thermo Fisher Scientific, Franklin, MA, USA). The analytical column used for the chromatographic separation was a Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA). The sample was loaded into enrichment columns using ultrapure water acidified at pH 4.5 with formic acid. After the enrichment step, the analytes were transferred to the analytical column for their chromatographic separation. The gradient used is shown in Table S2 (Supporting Information).

After separation, the detection of the selected analytes was accomplished by using a triple quadrupole mass spectrometer Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA), equipped with a Turbo Ion Spray source. All the analyses were performed operating in the negative electrospray ionization (ESI (−)) mode. Acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte (European Commission Decision 2002/657/EC). The main *m/z* transitions are summarized in Table S3 (Supporting Information). For analyte identification, the following conditions had to be met: i) analyte retention time in the sample must be in agreement with analyte retention time in the calibration curve; ii) two *m/z* transition were confirmed for every analyte; iii) ratio between the two transitions in the sample compared to ratio in the calibration curve should be in agreement to [calibration curve average ± SD (calibration curve)]. Table S4 (Supporting Information) provides the method limit of detection (MLOD) and the method limit of quantification (MLOQ) of the selected compounds in the five analyzed human tissues.

2.4. Quality assurance and quality control

To eliminate sources of contamination from the analytical system, all the polytetrafluoroethylene (PTFE) tubing was replaced by polyether ether ketone (PEEK) connections. In addition, an extra analytical column (C8 50 × 3 Thermo Scientific) was directly placed upstream of the injector to trap the instrumental sources of analytes, and therefore, to minimize the background signal and inter-run variability of all analytes. Blanks, consisting on initial conditions of mobile phase, were analyzed every 5 sample injections. For assessment of matrix interference in the analysis, matrix-matched calibration curves, and blank samples, were introduced in each run of analysis.

Spiking experiments were performed with blank animal (pig) matrices of brain, lung, liver, bone and kidney fortified at three different concentration levels (6, 12 and 24 ng/g of tissue). To assess the initial concentrations of PFASs, these samples were analyzed prior to fortification, being in all cases below the MLOD. The method was validated according to the criteria described by the EC Decision 2002/657/EC. The following parameters were established: instrumental selectivity and methodology limits of detection and quantification (ILOD, MLOD, ILOQ and MLOQ, respectively), linearity, recoveries, and precision expressed as intraday and inter-day repeatability.

2.5. Multivariate analysis

Before executing the multivariate data analysis, non-detected values were assumed to be equal to one-half of the method limit of detection (ND = 1/2 MLOD). The whole data set from the 5 human tissues was analyzed both individually and by using a column-wise 99 × 20 matrix augmentation strategy (Navarro et al., 2006). Auto scaling was chosen as pre-treatment method. With this procedure, the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance (Brodnjak-Vončina et al., 2002; Massart et al., 1998). Auto scaling can be applied either to the individual matrices corresponding to each tissue before matrix augmentation, or once they have been arranged in the column-wise augmented data matrix. The former system identifies differences in the tissues, while the latter detects differences among individual samples.

Data were also subjected to Principal Component Analysis (PCA). This is a data reduction technique aimed at explaining most of the variance in the data by transforming a set of correlated measured variables into a new set of uncorrelated Principal Components (PCs), which preserve the relationships present in the original data (Rovira et al., 2011a). The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships between objects and variables determined in large or complex data set (Rovira et al., 2011b). PCA can be easily extended to the simultaneous analysis of multiple correlated data sets. In the present study, PCA was conducted to assess the possible distribution of the different compounds in the tissues studied, as well as to assess any possible correlation between age and smoking habits of the subjects and their PFASs accumulations. PCA modelling was conducted using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under the MATLAB computer and visualization environment (The Mathworks, Natick, MA, USA). Finally, a hierarchical cluster analysis was conducted to confirm some of the conclusions obtained by the PCA. Data were also treated by normalization. The dissimilarity matrix was conducted by the Euclidean distance, while the Ward method was chosen for the aggragation approach. This part of the multivariate analysis was conducted using the XLSTAT module version 2012.042.

3. Results and discussion

The concentrations of detected PFASs in human samples of brain, liver, lung, bone and kidney are depicted in Fig. 1. The complete set of results of each one of the 99 analyzed samples is given in Table S5 (Supporting Information), while a summary of median and range values is presented in Table 1. All samples showed detectable values of at least two of the investigated compounds. Although PFASs accumulation followed different trends depending on the specific tissue, some similarities were observed between liver and brain, on one hand, and between kidney and lung, on the other hand. In liver, PFHxA, PFOS and FHEA were the most prevalent compounds, with median concentrations of 68.3, 41.9 and 16.7 ng/g, respectively. PFOS, one of the most toxic PFASs, was present in 90% of the samples, while PFOA could be quantified in 45% of the samples (median: 4.0 ng/g). In brain, PFHxA was the main compound, being detected in all the samples at concentrations ranging from 10.1 to 486 ng/g. The contributions of PFNA (median: 13.5 ng/g) and PFDA (median: 12.4 ng/g) were also relatively important in brain samples. In contrast, PFOS was only quantified in 20% of the samples (median: 1.9 ng/g), whereas PFOA was not detected in any of them. In general terms, lung was the tissue showing the highest accumulation of PFASs. PFBA and PFHxA were the compounds presenting the highest median concentrations (807 and 207 ng/g, respectively). Only two lung samples showed PFOS levels under the limit of detection, with a median value of 28.4 ng/g. Although the percentage of samples with detected values of PFOA fell down to 45%, the contribution of PFOA to the total PFASs in lung was quite important, in comparison to

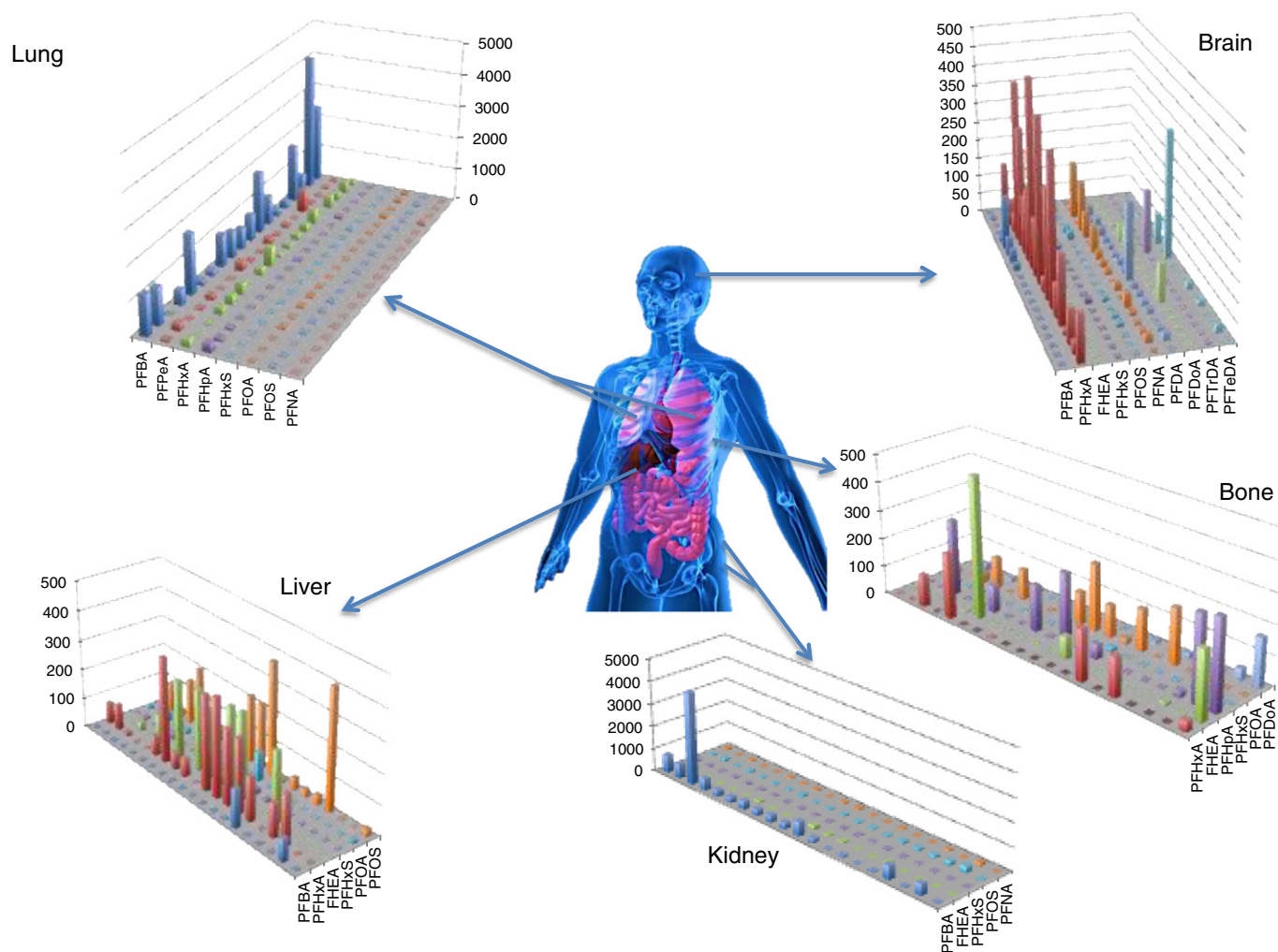


Fig. 1. Concentrations of various PFASs (in ng/g) in 5 human tissues from 20 residents of Tarragona (Catalonia, Spain).

other tissues and analytes. PFBA was also the predominant compound in all kidney samples, whose median concentration was 263 ng/g. PFDoDA and PFDA were also detected in kidney samples, but at much lower concentrations (median: 91.4 and 90.2 ng/g, respectively). High concentrations of PFOS were also found in kidney (median: 55.0 ng/g), while the presence of PFOA was minor. In contrast to lung, bone was identified as the tissue with the lowest burdens of PFASs. Furthermore, the PFAS profile was substantially different from those of the remaining tissues, as PFOA was, far the major contributor to the total concentration of PFASs (median: 20.9 ng/g). In turn, PFOS was not detected in any of the bone samples (Table 1). In summary, the profiles of PFASs accumulation in the different tissues reflected some common trends. Thus, PFHxA showed the highest concentrations in brain and liver, while PFBA presented the maximum median levels in kidney and lung, with PFOA as the predominant compound in bone. PFOS accumulated basically in lung, liver and kidney, while the levels of PFOS in bone and brain were very low. We hypothesized that since PFBA is a short chain compound, its predominance in lung could reflect the inhalation of contaminated dust and the industrial replacement of the eight carbons chain compounds by shorter ones. In addition, the human half-life of this compound is much shorter (3 days) (Chang et al., 2008) compared to the half-life to other longer chain compounds as those with 8 carbon-chain thereby accounting for its detection in other tissues as kidney. As aforementioned, there is an important lack of studies reporting PFASs levels in human tissues, excepting plasma. In comparison to previous results (Kärman et al., 2009; Maestri et al., 2006; Olsen et al., 2003), the current concentrations of PFOS in liver

from residents in Tarragona fall in the higher part of the range. However, this comparison can be only taken into account as a first indication.

The physical–chemical properties of each chemical are responsible for their tissue-specific accumulation profiles. However, the overall body burden can be similar although the chemicals accumulate in different tissues. In order to determine whether exposure to PFASs is related to exposures to other contaminants, the levels of PFASs in each sample were evaluated to determine whether they correlate with the concentrations of some metals and PCDD/Fs. The content of arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), and thallium (Tl) had been previously determined in the same human tissue samples (unpublished results). With a few exceptions, the levels of PFASs were not associated with those of most trace elements. However, a significant Pearson correlation was noted between PFOA and As ($p < 0.001$), as well as between PFOA and Pb ($p < 0.001$). Manganese was the element presenting a significant correlation with a higher number of PFASs: PFDS, PFUDA, and PFTeDA ($p < 0.001$ in all cases). Finally, Ni correlated with PFHxDA. However, PFOS did not correlate with any of the above elements (Table S6; Supporting Information). The concentrations of PCDD/Fs had been also analyzed in adipose tissues from 15 of the same 20 individuals (Nadal et al., 2009). The mean PCDD/F concentration in adipose tissue was 14.6 pg WHO-TEQ/g of fat (range: 3.3–55.4 pg WHO-TEQ/g of fat). The total levels of PCDD/Fs, as well as those of the 17 2,3,7,8-chlorinated congeners, were compared with the concentrations of PFASs accumulated in the 5 human tissues here analyzed. Although not statistically significant, a negative correlation was

Table 1
Summary of PFAS concentrations (in ng/g wet weight) in 5 autopsy tissues from 20 individuals of Tarragona (Catalonia, Spain).

	Liver				Bone				Brain				Lung				Kidney			
	Mean	Median	Range	MILOD	% of detection	Mean	Median	Range	MILOD	% of detection	Mean	Median	Range	MILOD	% of detection	Mean	Median	Range	MILOD	% of detection
PFBA	12.9	3.0	128-Bdl.	6.00	10	Bdl.	–	–	0.03	0	13.5	1.4	137-Bdl.	2.71	25	304.2	807	4138-Bdl.	0.01	95
PFPeA	1.4	Bdl.	27.1-Bdl.	0.001	5	0.8	0.8	0.8-Bdl.	1.51	0	Bdl.	–	–	0.59	0	44.5	40.8	695-Bdl.	6.006	74
PFBS	0.9	0.7	1.5-Bdl.	1.39	0	3.2	2.4	17.8-Bdl.	14.41	5	Bdl.	–	–	0.96	0	17.8	1.1	9.7-Bdl.	2.10	47
PFHxA	11.5	68.3	353-Bdl.	2.73	70	35.6	1.5	230-Bdl.	0.001	30	180	141	486–10.1	0.72	100	50.1	207	569-Bdl.	9.42	89
FHEA	92.6	16.7	289-Bdl.	4.40	45	42.5	2.0	494-Bdl.	3.52	25	18.6	2.0	93.1-Bdl.	4.00	25	2.4	3.9	3.9-Bdl.	5.54	0
PFHpA	33.3	1.5	638-Bdl.	3.00	5	77.1	2.4	309-Bdl.	2.89	45	Bdl.	–	–	2.70	0	17.4	1.5	245-Bdl.	3.00	37
PFHxS	4.6	1.8	20.6-Bdl.	3.00	10	1.8	1.2	13.8-Bdl.	2.40	5	3.2	2.3	14.4-Bdl.	4.54	5	8.1	5.7	47.6-Bdl.	3.30	32
PFOA	13.6	4.0	98.9-Bdl.	3.00	45	60.2	20.9	234-Bdl.	3.00	55	Bdl.	–	–	2.40	0	29.2	12.1	87.9-Bdl.	6.00	42
PFOS	102	41.9	405-Bdl.	3.00	90	Bdl.	–	–	3.00	0	4.9	1.9	22.5-Bdl.	3.00	20	29.1	28.4	61.8-Bdl.	3.00	89
PFNA	1.3	1.0	6.6-Bdl.	1.99	0	Bdl.	–	–	4.18	0	29.7	13.5	150-Bdl.	3.27	55	15.3	3.5	126-Bdl.	7.13	11
FOEA	2.8	2.8	2.8-Bdl.	5.67	0	3.6	1.6	35.7-Bdl.	3.20	5	Bdl.	–	–	8.80	0	13.2	4.9	87-Bdl.	5.60	21
PFODA	2.5	1.5	6.5-Bdl.	3.00	0	Bdl.	–	–	6.00	0	Bdl.	–	–	2.91	0	Bdl.	–	–	2.91	0
PFDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.30	0	23.4	12.4	204-Bdl.	2.94	70	17.1	1.5	108-Bdl.	2.973	32
PFOA	Bdl.	–	–	2.60	0	Bdl.	–	–	2.04	0	Bdl.	–	–	2.04	0	Bdl.	–	–	10.16	0
PFDS	Bdl.	–	–	0.001	5	1.7	1.5	5.7-Bdl.	2.97	0	0.3	Bdl.	1.4-Bdl.	0.00	25	3.1	0.6	9-Bdl.	1.200	37
PFUdA	Bdl.	–	–	0.003	0	Bdl.	–	–	0.30	0	Bdl.	–	–	18.00	0	2.8	1.4	20.4-Bdl.	2.700	11
FDEA	3.7	0.7	59.3-Bdl.	3.00	5	Bdl.	–	–	0.30	0	Bdl.	–	–	2.91	0	Bdl.	–	–	0.01	0
PFDOA	2.4	1.5	20.2-Bdl.	1.45	5	16.6	5.1	169-Bdl.	0.98	70	13.2	1.5	102-Bdl.	1.32	25	20.7	Bdl.	253-Bdl.	4.76	11
PFTDA	2.1	Bdl.	32-Bdl.	0.001	10	15.8	0.3	311-Bdl.	0.60	5	9.9	1.4	167-Bdl.	2.88	10	138.6	6.9	1582-Bdl.	2.970	42
PFTeDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.001	5	24.8	1.4	335.7-Bdl.	2.85	30	9.8	1.5	82.8-Bdl.	2.910	16
PFHxDA	Bdl.	–	–	3.00	0	16.6	2.9	171.8–2.9	5.85	10	Bdl.	–	–	2.91	0	8.5	1.5	80.2-Bdl.	2.95	16

MILOD: Method Limit of Detection. Bdl.: Below limit of detection.

observed between the total sum of PCDD/Fs and the total amount of PFASs (Table S7; Supporting Information). It is well known that the toxicity of dioxins is mediated through the activation of the Aryl hydrocarbon Receptor (AhR) (White and Birnbaum, 2009). In contrast, the mode-of-action (MoA) for PFOA as well as other PFASs, is not so well understood (Post et al., 2012). Notwithstanding, it must be noted that data on PCDD/Fs were only available for adipose tissue, while PFASs levels refer to another 5 different tissues (liver, brain, kidney, bone, and lung). Therefore, these data are not entirely comparable, and consequently, this indication cannot be confirmed.

The pharmacokinetic properties of PFOA and PFOS are well studied (Loccisano et al., 2012). These parameters have been used in the development of pharmacokinetic models, aimed at describing the human distribution of PFOA and PFOS (Loccisano et al., 2011; Thompson et al., 2010), among other PFASs. Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the human body, where organs are considered as compartments (Fàbrega et al., 2011). The overall goal of developing these PBPK models is to extrapolate to humans the distribution of chemicals in the body, in order to enhance the scientific basis for human health risk assessment of PFASs (Loccisano et al., 2012). According to the results of studies with experimental animals, these compounds are well absorbed orally (Loccisano et al., 2012). Therefore, ingestion should be considered a key pathway. A clear relationship between the intake of PFOA, basically through drinking water consumption, and serum concentrations in humans, has been found (Emmett et al., 2006), with a with a serum:drinking water ratio of about 100:1 (Post et al., 2012). Although a number of PBPK models have been described, most of them have been based only on animal data, while human data are still very scarce. To the best of our knowledge, we here report, for the very first time, the simultaneous accumulation of PFASs in various human tissues. This information should be beneficial for the development of theoretical PBPK models, whose validation is still incomplete. Consequently, forensic analyses offer a practical way to explore the real accumulation of those pollutants in the human body.

In the current study, PCA analysis was used to determine the variation of PFASs accumulation between tissues, as well as to extract possible relations between the individual concentrations and other factors, such as age and smoking. The PCA results are summarized in Table 2. The first PC explained a variance ranging between 12% and 29% of the total variance, for all the different tissues analyzed, while PC2 and PC3 variances ranged 19–20% and 8–15% respectively. The percentage of explained variance for those PCAs performed in the individual tissues was always higher than that in the augmented matrices. The explained variances differed in the two groups of PCAs. In the augmented matrices, they increased very slowly, not reaching 50% of the total variance until PC6. This indicates the presence of multiple independent distribution processes of PFASs in the considered tissues. On the other hand, in the individual PCAs of each tissue, the variance increased faster, reaching 50% of the total variance in the PC3 in most of the cases, indicating similar distribution processes when the same tissue is considered. Fig. 2 depicts the loadings plot for the first two PCs of the augmented and auto scaled data matrices. The first PC had positive loadings for all acidic compounds, from low to high contributions depending on the compound, except for PFHxA, PFHpA, with moderate negative loadings, and PFDOA, with a high negative loading. In this first PC, perfluoroalkyl sulphonates presented positive loadings, with higher contributions of PFHxS and PFDS. Regarding telomer acids (FHEA, FDEA and FOEA), the three compounds showed moderate loadings, negatively for FHEA and FDEA, and positively for FOEA. The second PC showed positive loadings for most acidic compounds except for PFNA, PFDA, PFUdA and PFTeDA, with especially high contributions of PFBA, PFOA, PFDA and PFTeDA. Perfluoroalkyl sulphonates presented moderate contributions to the second PC, being positive for PFBS and PFDS, and negative for the remaining two. The telomer acids presented positive loadings for FOEA and FDEA, and negative for FHEA. When plotting

Table 2

Percentages of explained variances obtained by PCAs applied to All_{aug-auto}, All_{auto-aug} and the individual matrices of the 5 tissues.

Matrix	All _{aug-auto}	All _{auto-aug}	Liver	Brain	Bone	Lung	Kidney
PC1	11.98	12.49	28.68	22.69	25.80	18.65	20.20
PC2	9.26	11.23	19.91	17.10	16.18	16.28	14.75
	(21.25)	(23.72)	(48.59)	(39.79)	(41.98)	(34.93)	(34.96)
PC3	7.65	9.82	15.50	13.68	13.64	12.55	14.56
	(28.90)	(33.53)	(64.09)	(53.47)	(55.63)	(47.48)	(49.51)
PC4	7.49	8.28	9.78	10.96	12.52	9.66	12.17
	(36.39)	(41.81)	(73.87)	(64.43)	(68.15)	(57.14)	(61.69)
PC5	6.75	7.53	8.53	9.30	8.20	8.02	8.75
	(43.14)	(49.35)	(82.40)	(73.73)	(76.35)	(65.16)	(70.43)
PC6	6.47	6.22	5.47	7.55	6.95	7.74	8.26
	(49.61)	(55.57)	(87.87)	(81.28)	(83.30)	(72.90)	(78.70)
PC10	4.60	4.55	1.07	2.07	1.79	3.58	2.82
	(70.28)	(76.06)	(99.74)	(98.01)	(100)	(91.42)	(95.92)

In parenthesis, percentage of accumulated variance for that particular component.

All_{aug-auto}: augmented matrix of the 5 individual tissues and then autoscaled.

All_{auto-aug}: individually autoscaled matrixes of the 5 tissues and then augmented.

the scores using these two PCs, the samples can be grouped into each one of the 5 tissues analyzed (Fig. 3). This means that the profile of PFASs found in each tissue is different from the others. Thus, PC1 allowed the separation between lungs, kidney and brain, with positive contribution, while bone and liver showed a negative contribution. In turn, PC2 reflected a separation between lung and bone, with positive loadings, and the remaining three tissues, with negative loadings. When considering the remaining PCs, the behavior was similar.

Fig. S1 (Supporting Information) depicts the loadings plot for the first PC of each PCA performed in the individual matrices of each tissue. In liver, the first PC showed high positive loadings for acidic compounds with an odd number of carbons (PFPeA, PFHpA, PFNA and

PFTTrDA). In kidney, a similar profile was obtained, with acidic PFASs with an odd number of carbon chain (PFPeA, PFNA, PFUDa and PFTTrDA) acting as prevalent compounds. In brain, acidic compounds with a pair number of carbon chain (PFBA and PFHxA) and sulphonates (PFBS, PFOS and PFDS) were the predominant compounds. Unlike other PFASs, sulphonates also showed a high contribution in bone. Lung samples also presented positive loadings for most acidic compounds with a pair number of carbon chain (PFBA, PFHxA, PFOA, PFDoA), as well as some of the sulphonates (PFOS, PFDS) and FOEA. This different profile of PC1 confirms the different distribution pattern of PFASs according to each specific tissue. The influence of smoking in the accumulation of PFAs in the lungs was also studied. As shown in Fig. 4, smoker subjects presented lower contributions of PC1 and PC2 than non-smokers. It means less accumulation of the PFASs, which contribute to these PCs. When considering the rest of PCs, a similar behavior is observed. Considering the samples included in this study, a negative correlation between smoking habits and accumulations of PFAs in lung is observed. Further investigation with a higher number of subjects should be performed to check this relationship.

The accumulation of PFASs with age was studied in the analyzed tissues. In general terms, older people (more than 60 years) showed higher concentrations of PFASs, which is a clear indication that these compounds accumulate after a long-term exposure. All middle-age (40–60 years) individuals presented fairly similar levels of PFASs in the different tissues. However, some young subjects (18–39 years) also showed relatively high levels of PFASs. These values could be due to differential accumulation factors, such as dietary intake, living habits, and/or early exposure. This was also confirmed after performing a hierarchical classification (Fig. S2). Finally, a special correlation between smoking habits and PFAS accumulation in lung was performed. Although PCs did not show a positive relation between both

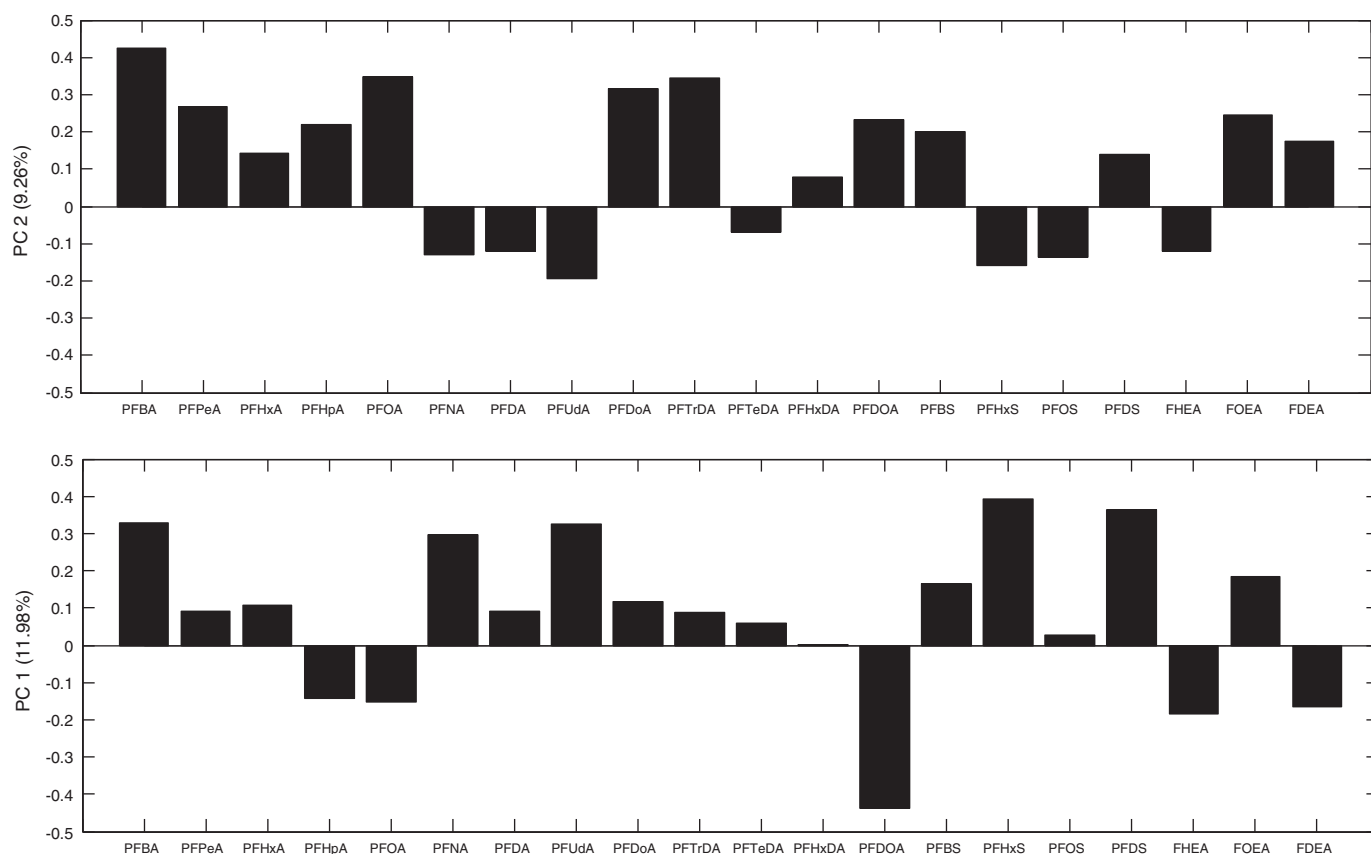
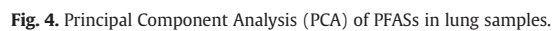


Fig. 2. Loadings of the first two principal components (PCs) for the augmented and auto scaled matrices.



parameters, the current number of samples was not sufficient to establish conclusions on this issue. Further investigations involving a higher number of subjects are necessary.

4. Conclusions

In this study, an effective analytical method optimized for the ultra-trace analysis of 21 PFASs in human tissues, using both small sample sizes (amount: 1 g) and a reduced sample manipulation, was addressed. The application of this approach to the analysis of 99 samples of five different tissues from 20 subjects demonstrated, for the very first time, the accumulation of certain short chain compounds, such as PFBA and PFHxA, in human tissues. Moreover, the results from the chemical analysis, together with the application of multivariate statistical techniques, showed a different accumulation pattern of the analyzed compounds in human tissues. Only few correlations were noted in the concentrations of metals and those of PFASs. However, interestingly, certain negative association between the contents of PFASs in those 5 autopsy tissues, and the levels of PCDD/Fs in adipose tissue, was observed. This finding suggests the need to fully characterize the toxicity mechanisms of PFASs, which are not currently so well understood as those of PCDD/Fs. Notwithstanding, as data refer to different biological compartments, values are not entirely comparable. In any case, the current results should be of importance for the validation of PBPK models, which are being developed for humans.

Acknowledgments

This work was supported by the Spanish Ministry of Science and Innovation, through the project SCARCE Consolider Ingenio 2010 CSD2009-00065 and by King Saud University grant number (KSU-VPP-105). This work was partly supported by the Generalitat de Catalunya (Consolidated Research Groups: Water and Soil Quality Unit 2009-SGR-965 and Laboratory of Toxicology and Environmental Health 2009-SGR-1133). Thermo Company is acknowledged for providing free columns.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.envint.2013.06.004>.

References

- Brodjak-Vončina D, Dobčnik D, Novič M, Zupan J. Chemometrics characterisation of the quality of river water. *Anal Chim Acta* 2002;462:87–100.
- Chang SC, Das K, Ehresman DJ, Ellefson ME, Gorman GS, Hart JA, et al. Comparative pharmacokinetics of perfluorobutylate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. *Toxicol Sci* 2008;104:40–53.
- Cui L, Liao CY, Zhou QF, Xia TM, Yun ZJ, Jiang GB. Excretion of PFOA and PFOS in male rats during a subchronic exposure. *Arch Environ Contam Toxicol* 2010;58:205–13.
- Domingo JL, Ericson-Jogsten I, Perelló G, Nadal M, van Bavel B, Kärrman A. Human exposure to perfluorinated compounds in Catalonia, Spain: contribution of drinking water and fish and shellfish. *J Agric Food Chem* 2012a;60:4408–15.
- Domingo JL, Ericson-Jogsten I, Eriksson U, Martorell I, Perelló G, Nadal M, et al. Human dietary exposure to perfluoroalkyl substances in Catalonia Spain. *Temporal trend. Food Chem* 2012b;135:1575–82.
- EFSA. Opinion of the Scientific Panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and its salts (question no. EFSA-Q-2004-163). *EFSA J* 2008;6:53:1–131.
- Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. *J Occup Environ Med* 2006;48:759–70.
- Ericson I, Domingo JL, Nadal M, van Bavel B, Lindström G. Levels of perfluorochemicals in water samples from Catalonia, Spain: is drinking water a significant contribution to human exposure? *Environ Sci Pollut Res* 2008;15:614–9.
- Ericson I, Domingo JL, Nadal M, Bigas E, Llebaria X, van Bavel B, et al. Levels of perfluorinated chemicals in municipal drinking water from Catalonia, Spain: public health implications. *Arch Environ Contam Toxicol* 2009;57:631–8.
- Ericson I, Gómez M, Nadal M, van Bavel B, Lindström G, Domingo JL. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: a pilot study. *Environ Int* 2007;33:616–23.
- Ericson-Jogsten I, Nadal M, van Bavel B, Lindström G, Domingo JL. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: implications for human exposure. *Environ Int* 2012;39:172–80.
- Fàbrega F, Nadal M, Schuhmacher M, Domingo JL. PBPK modeling of PFOS and PFOA using a whole body pharmacokinetic model: a preliminary approach and validation test. *Organohalogen Compd* 2011;73:1169–72.
- Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, et al. Pre- and postnatal exposure to perfluorinated compounds (PFCs). *Environ Sci Technol* 2010;44:7123–9.
- Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res* 2005;99:253–61.
- Haug LS, Thomsen C, Becher G. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. *J Chromatogr A* 2009a;1216:385–93.
- Haug LS, Thomsen C, Becher G. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ Sci Technol* 2009b;43:2131–6.
- Johansson N, Eriksson P, Viberg H. Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. *Toxicol Sci* 2009;108:412–8.
- Johansson N, Fredriksson A, Eriksson P. Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. *Neurotoxicol* 2008;29:160–9.
- Jones PD, Hu W, de Coen W, Newsted JL, Giesy JP. Binding of perfluorinated fatty acids to serum proteins. *Environ Toxicol Chem* 2003;22:2639–49.
- Kärrman A, Harada KH, Inoue K, Takasuga T, Ohi E, Koizumi A. Relationship between dietary exposure and serum perfluorochemical (PFC) levels—a case study. *Environ Int* 2009;35:712–7.
- Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol Sci* 2007;99:366–94.
- Lindstrom AB, Strynar MJ, Libelo EL. Polyfluorinated compounds: past, present, and future. *Environ Sci Technol* 2011;45:7954–61.
- Llorca M, Farré M, Picó Y, Teijón ML, Álvarez JG, Barceló D. Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. *Environ Int* 2010;36:584–92.
- Loccisano AE, Campbell JL, Andersen ME, Clewell HJ. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. *Regul Toxicol Pharmacol* 2011;59:157–75.
- Loccisano AE, Campbell Jr JL, Butenhoff JL, Andersen ME, Clewell III HJ. Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. *Reprod Toxicol* 2012;33:452–67.
- Loi EIH, Yeung LWY, Taniyasu S, Lam PKS, Kannan K, Yamashita N. Trophic magnification of poly- and perfluorinated compounds in a subtropical food web. *Environ Sci Technol* 2011;45:5506–13.
- Luebker DJ, Hansen KJ, Bass NM, Butenhoff JL, Seacat AM. Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* 2002;176:175–85.
- Maestri L, Negri S, Ferrari M, Ghittori S, Fabris F, Danesino P. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. *Rapid Commun Mass Spectrom* 2006;20:2728–34.
- Maisonet M, Terrell ML, McGeehin MA, Christensen KY, Holmes A, Calafat AM, et al. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. *Environ Health Perspect* 2012;120:1–26.
- Massart D, Vandeginste M, Buydens L, de Jong S, Lewi P, Smeyers-Verbeke J. Handbook of chemometrics and qualimetrics: Part B. Amsterdam: Nederland: Elsevier; 1998.
- Nadal M, Domingo JL, García F, Schuhmacher M. Levels of PCDD/F in adipose tissue on non-occupationally exposed subjects living near a hazardous waste incinerator in Catalonia, Spain. *Chemosphere* 2009;74:1471–6.
- Navarro A, Tauler R, Lacorte S, Barceló D. Chemometrical investigation of the presence and distribution of organochlorine and polycyclic aromatic compounds in sediments of the Ebro River Basin. *Anal Bioanal Chem* 2006;385:1020–30.
- Olsen GW, Burris JM, Ehresman DJ, Froelich JW, Seacat AM, Butenhoff JL, et al. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect* 2007;115:1298–305.
- Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH. Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. *Environ Sci Technol* 2003;37:888–91.
- Pirali B, Negri S, Chytiris S, Perissi A, Villani L, La Manna L, et al. Perfluorooctane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. *Thyroid* 2009;19:1407–12.
- Post GB, Cohn PD, Cooper KR. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. *Environ Res* 2012;116:93–117.
- Post GB, Louis JB, Cooper KR, Boros-Russo BJ, Lippincott RL. Occurrence and potential significance of perfluorooctanoic acid (PFOA) detected in New Jersey public drinking water systems. *Environ Sci Technol* 2009;43:4547–54.
- Powley CR, George SW, Russell MH, Hoke RA, Buck RC. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. *Chemosphere* 2008;70:664–72.

- Rovira J, Mari M, Nadal M, Schuhmacher M, Domingo JL. Levels of metals and PCDD/Fs in the vicinity of a cement plant: assessment of human health risks. *J Environ Sci Health A* 2011a;46:1075–84.
- Rovira J, Mari M, Schuhmacher M, Nadal M, Domingo JL. Monitoring environmental pollutants in the vicinity of a cement plant: a temporal study. *Arch Environ Contam Toxicol* 2011b;60:372–84.
- Schechter A, Malik-Bass N, Calafat AM, Kato K, Colacino JA, Gent TL, et al. Polyfluoroalkyl compounds in Texas children from birth through 12 years of age. *Environ Health Perspect* 2012;120:590–4.
- Thompson J, Lorber M, Toms LML, Kato K, Calafat AM, Mueller JF. Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. *Environ Int* 2010;36:390–7.
- Vanden Heuvel JP, Kuslikis BI, Peterson RE. Covalent binding of perfluorinated fatty acids to proteins in the plasma, liver and testes of rats. *Chem Biol Interact* 1992;82:317–28.
- Viberg H, Lee I, Eriksson P. Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. *Toxicology* 2013;304:185–91.
- Vieira VM, Hoffman K, Shin HM, Weinberg JM, Webster TF, Fletcher T. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. *Environ Health Perspect* 2013;121:318–23.
- Weiss JM, Andersson PL, Lamoree MH, Leonards PEG, Van Leeuwen SPJ, Hamers T. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicol Sci* 2009;109:206–16.
- White SS, Birnbaum LS. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J Environ Sci Health C* 2009;27:197–211.
- Zhao YG, Wong CKC, Wong MH. Environmental contamination, human exposure and body loadings of perfluorooctane sulfonate (PFOS), focusing on Asian countries. *Chemosphere* 2012;89:355–68.